

Amendments to the Specification:

Please replace the paragraph beginning on page 1 at line 7 with the following amended paragraph:

Nuclear factor κ B (NF- κ B) is a family of inducible eukaryotic transcription factor complexes participating in regulation of immune response, cell growth, and survival [Ghosh et al. 1998]. The NF- κ B factors are normally sequestered in the cytoplasmic compartment by physical association with a family of cytoplasmic ankyrin rich inhibitors termed I κ B, including I κ B α and related proteins [Baldwin et al. 1996]. In response to diverse stimuli, including cytokines, mitogens, and certain viral gene products, I κ B is rapidly phosphorylated at serines 32 and 36, ubiquitinated and then degraded by the 26S proteasome, which allows the liberated NF- κ B to translocate to the nucleus and participate in target gene transactivation [Mercurio et al 1999, Pahl et al 1999]. Recent molecular cloning studies have identified a multi subunit I κ B kinase (IKK) that mediates the signal-induced phosphorylation of I κ B. The IKK is composed of two catalytic subunits, IKK α and IKK β , and a regulatory subunit IKK γ . The catalytic activity of both IKK α and IKK β can be activated by a multitude of different NF- κ B inducers, including the inflammatory cytokines, tumor necrosis factor

and interleukin-1, the T cell receptor and the T cell costimulatory protein, CD28 [Karin et al 2000].

Please replace the paragraph beginning on page 3 at line 13 with the following amended paragraph:

Assessment of the pattern of the NF- κ B species in lymphoid organs of *aly/aly* mice indicated that, apart from its role in the regulation of NF- κ B complex(s) comprised of Rel proteins (A+p50) and I κ B, NIK also participates in controlling the expression/activation of other NF- κ B species. Most notably, the lymphocytes of the *aly/aly* mice were deficient of p52, an NF- κ B species that is specifically formed in mature B-lymphocytes through proteolytic processing of an inactive precursor, p100 (NF- κ B2), suggesting a deficiency in p100 - p52 conversion [Yamada et al. 2000]. Indeed, NIK has been shown to participate in site specific phosphorylation of p100. ~~Both both directly and through~~ phosphorylation of IKK α , which in turn phosphorylates p100. This phosphorylation serves as a molecular trigger for ubiquitination and active processing of p100 to form p52. This p100 processing activity was found to be ablated by the *aly* mutation [Xiao et al. 2001, Senftleben et al. 2001].

Please replace the paragraph beginning on page 4 at line 7 with the following amended paragraph:

Like other MAP3Ks, NIK can be activated as a consequence of phosphorylation of the 'activation loop' within the NIK molecule. Indeed, mutation of a phosphorylation-site within this loop (Thr-559) prevents activation of NF- κ B upon NIK overexpression [Lin et al. 1999]. In addition, the activity of NIK seems to be regulated through the ability of the regions upstream and downstream of its kinase motif to bind to each other. The C-terminal region of NIK downstream of its kinase moiety has been shown to be capable of binding directly to IKK α [Regnier et al. 1997] as well as to p100 [Xiao et al. 2001] and to TRAF2 [Malinin et al. 1997]. These interactions are apparently required for NIK function in NF- κ B signaling. The N-terminal region of NIK contains a negative-regulatory domain (NRD), which is composed of a basic motif (BR) and a proline-rich repeat motif (PRR) [Xiao et al. 2000]. Apparently, the N-terminal NRD interacts with the C-terminal region of NIK in cis, thereby inhibiting the binding of NIK to its substrate (IKK α and p100). Ectopically expressed NIK seems to spontaneously form oligomers in which these bindings of the N-terminal to the C-terminal regions in each NIK molecule are apparently disrupted, and display a high level of constitutive activity [Lin et al. 1999]. The binding

of the NIK C-terminal region to TRAF2 (as well as to other TRAF's) most likely participates in the activation process of NIK. However, its exact mode of participation is unknown.

Please replace the paragraph beginning on page 4 at line 25 with the following amended paragraph:

There is likewise rather limited information yet of the downstream mechanisms in NIK action. Evidence has been presented that NIK, through the binding of its C-terminal region to IKK α can activate the I κ B kinase (IKK) complex. It has indeed been shown to be capable of phosphorylating serine-176 in the activation loop of IKK α , ~~and its activation thereby activating IKK α~~ [Ling et al. 1998]. ~~Consistently~~ Consistent with such mode of action, studies of the mechanisms accounting ~~to~~ for the deficient activation of NF- κ B by the LT β R in *aly/aly* mice murine embryonic fibroblasts (MEF's) indicated that NIK mutation ablates activation of the IKK signalosome and the consequent phosphorylation of I κ B [Matsushima et al 2001]. These findings were not supported, however, by the analysis of MEF's derived from NIK -/- mice. Although the NIK deficient MEF's are unable to manifest NF- κ B activation in response to LT β , they do seem to respond normally to it in terms of I κ B phosphorylation and degradation [Yin et al.

2001]. According to these findings, NIK may not participate at all in the activation of the IKK complex by the LT β R but is rather involved by an as yet unknown mechanism in controlling the transcriptional action of the NF- κ B complex after its translocation to the nucleus. There are also still uncertainties as to the way by which NIK triggers p100 phosphorylation and processing. Its ability to bind p100 directly through its C-terminal region and phosphorylate it suggests that p100 serves as a direct NIK substrate [Xiao et al. 2000]. Nevertheless, a recent study has suggested that NIK mediates p100 phosphorylation in an indirect way, through phosphorylation and thus activation of IKK α that in turn phosphorylates p100 [Senftleben et al. 2001].

Please replace the paragraph beginning on page 7 at line 23 with the following amended paragraph:

Mouse and human IL2 both cause proliferation of T-cells of the homologous species at high efficiency. Human IL2 also stimulates proliferation of mouse T-cells at similar concentrations, whereas mouse IL2 stimulates human T-cells at a lower (sixfold to 170-fold) efficiency. The involvement of IL-2 in autoimmunity is controversial (reviewed by O'Shea et al. 2002) It is recognized that IL-2 administration is associated with a variety of autoimmune disorders such as

immune thyroiditis, rheumatoid arthritis and other
~~arthropaties~~ arthropathies. However IL-2 deficient mice
produce multiple autoantibodies, including anti-DNA
antibodies. About half die of autoimmune haemolytic anemia and
the survivors develop inflammatory bowel disease. Importantly,
the pathology is corrected by the addition of exogenous IL-2.
This indicates a role of IL-2 in maintaining peripheral
tolerance.

Please replace the paragraph beginning on page 8 at
line 5 with the following amended paragraph:

IL2 is a growth factor for all subpopulations of T-
lymphocytes. The IL2R-alpha receptor subunit is expressed in
adult T-cell leukemia (ATL). Since freshly isolated leukemic
cells also secrete IL2 and respond to it, IL2 may function as
an autocrine growth modulator for these cells capable of
worsening ATL.

Please replace the paragraph beginning on page 9 at
line 1 with the following amended paragraph:

Murine and human gamma subunits of the receptor have
approximately 70 percent sequence identity at the nucleotide
and amino acid levels. This subunit is required for the
generation of high and intermediate affinity IL2 receptors but

does not bind IL2 by itself. These two receptor types consist of an alpha-beta-gamma heterotrimer and a beta-gamma heterodimer, respectively. The gene encoding the gamma subunit of the IL2 receptor maps to human chromosome Xq13, spans approximately 4.2 kb and contains eight exons. Relationships to markers in linkage studies suggest that this gene and SCIDX1, the gene for X-linked severe combined immunodeficiency, have the same location. Moreover, in each of 3 unrelated patients with X-linked SCID, a different mutation in the IL2R-gamma gene has been observed.

Please replace the paragraph beginning on page 9 at line 11 with the following amended paragraph:

X-linked severe combined immunodeficiency (XSCID) is a rare and potentially fatal disease caused by mutations of IL2R γ chain, the gene encoding the IL-2R γ chain, a component of multiple cytokine receptors that are essential for lymphocyte development and function (Noguchi et al. 1993). To date, over 100 different mutations of IL2RG resulting in XSCID have been published. Recent gene knock out studies indicate a pivotal role of ~~the~~ eye this gene in lymphopoiesis [DiSanto et al 1995].

Please replace the paragraph beginning on page 10 at line 2 with the following amended paragraph:

The present invention relates to the use of NIK or a mutein, variant, fusion protein, functional derivative, circularly permuted derivative or fragment thereof, preferably the fragments comprised in SEQ ID NO:19, SEQID NO: 18, and the mutant AlyNIK, for modulating the interaction between IL-2 ~~common~~ receptor gamma chain (also known as common gamma chain) (cyc) and NIK and preferably to the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and to the mutant AlyNIK.

Please replace the paragraph beginning on page 10 at line 7 with the following amended paragraph:

In addition the invention relates to the use of a DNA encoding NIK according to the invention or its antisense, NIK specific antibodies, a small molecule obtainable by screening products of combinatorial chemistry in a luciferase system, for modulating the interaction between ~~IL-2~~ common gamma chain (cyc) and NIK.

Please replace the paragraph beginning on page 10 at line 11 with the following amended paragraph:

In another aspect, the present invention relates to the use of NIK or a mutein, variant, fusion protein,

functional derivative, circularly permuted derivative or fragment thereof, preferably the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and the mutant AlyNIK, in the manufacture of a medicament for the treatment of a disease, wherein a cytokine stimulating signalling ~~trough~~ through the ~~IL-2~~-cyc is involved in the pathogenesis of the disease.

Please replace the paragraph beginning on page 10 at line 16 with the following amended paragraph:

In addition the invention relates to the use of a DNA encoding NIK according to the invention or its antisense-, NIK specific antibodies, a small molecule obtainable by screening products of combinatorial chemistry in a luciferase system, for the treatment of a disease, wherein a cytokine stimulating signalling ~~trough~~ through the ~~IL-2~~-cyc is involved in the pathogenesis of the disease, for inhibiting signalling ~~trough~~ through ~~IL-2~~-cyc.

Please replace the paragraph beginning on page 10 at line 22 with the following amended paragraph:

The invention also provides methods for the treatment of a disease involving signalling of a cytokine ~~trough~~ through ~~IL-2~~-cyc in the pathogenesis of said disease, particularly IL-2 or IL-15, comprising administration of a

therapeutically effective amount of NIK or a mutein, variant, fusion protein, functional derivative, circularly permuted derivative or fragment thereof, preferably the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and the mutant AlyNIK, in a subject in need.

Please replace the paragraph beginning on page 10 at line 28 with the following amended paragraph:

In addition the invention relates methods for the treatment of a disease involving signalling of a cytokine ~~through~~ through IL-2 cyc in the pathogenesis of said disease comprising administration of a therapeutically effective amount of DNA encoding NIK according to the invention or its antisense, NIK specific antibodies or a small molecule obtainable by screening products of combinatorial chemistry in a luciferase system.

Please replace the paragraph beginning on page 11 at line 4 with the following amended paragraph:

Moreover, the present invention relates to a pharmaceutical composition comprising NIK or a mutein, variant, fusion protein, functional derivative, circularly permuted derivative or fragment thereof, ~~—~~ preferably the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and the

mutant AlyNIK, for modulating the interaction between ~~IL-2~~
common gamma chain (cyc) and NIK, wherein a cytokine
stimulating ~~IL-2~~-cyc signalling is involved in the
pathogenesis of the disease, a cytokine stimulating signalling
~~through~~ through ~~IL-2~~-cyc is involved in the pathogenesis of the
disease or the interaction between cyc and NIK is involved in
the pathogenesis of a disease.

Please replace the paragraph beginning on page 11 at
line 12 with the following amended paragraph:

In addition the invention relates to a
pharmaceutical composition comprising a DNA encoding NIK
according to the invention or its antisense, NIK specific
antibodies or a small molecule obtainable by screening
products of combinatorial chemistry in a luciferase system,
for the treatment of a disease, wherein a cytokine stimulating
signalling ~~through~~ through the ~~IL-2~~-cyc is involved in the
pathogenesis of the disease, for modulating the interaction
between ~~IL-2~~-common gamma chain (cyc) and NIK, wherein a
cytokine stimulating ~~IL-2~~-cyc signalling is involved in the
pathogenesis of the disease, a cytokine stimulating
signalling ~~through~~ through ~~IL-2~~-cyc is involved in the
pathogenesis of the disease or the interaction between cyc and
NIK is involved in the pathogenesis of a disease.

Please replace the paragraph beginning on page 11 at line 21 with the following amended paragraph:

In another aspect, the present invention relates to a polypeptide fragment of NIK, comprising the ~~IL-2R~~-common gamma chain (cyc) binding domain, or a mutein, variant, fusion protein, functional derivative, circularly permuted derivative or fragment thereof, preferably the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and the mutant AlyNIK , for modulating the interaction between ~~IL-2~~-common gamma chain (cyc) and NIK and preferably to the fragments comprised in SEQ ID NO:19, SEQ_ID NO: 18, and to the mutant AlyNIK, a DNA encoding the polypeptides of the invention, a vector comprising the DNA, host cells comprising the vector and a method for the production of a NIK polypeptide fragment according to the invention the cell of the invention and collecting the polypeptide produced.

Please replace the paragraph beginning on page 12 at line 6 with the following amended paragraph:

In addition the invention relates to a pharmaceutical composition comprising a polypeptide fragment of NIK, preferably the fragments comprised in SEQ ID NO:19, and in SEQ_ID NO: 18, DNA encoding the fragments or antisense

DNA, a vector comprising the DNA, antibodies of the invention or the small molecule able to inhibit NIK-cyc interaction obtainable by screening of molecules prepared by combinatorial chemistry in a luciferase system.

Please replace the paragraph beginning on page 15 at line 23 with the following amended paragraph:

Figure 6 shows the effect of cyc on NF- κ B activation induced by the NIK^Δ mutant. Activation of NF- κ B is monitored by the luciferase reporter assay (for details see Example 10). NF- κ B activation in cells is induced by overexpressing NIK. Luciferase expression was monitored in 293-T cells transfected with the following plasmids: empty plasmid (sample pc), empty plasmid and a plasmid encoding luciferase under the control of an NF- κ B inducible promoter (pcDNA3luciferase) (sample pc+luc), pcS3MTNIK and pcDNA3luciferase (sample NIK), pcDNA3luciferase and pcDNA3cyc (sample ~~cgc~~ cyc), pcS3MTNIK, pcDNA3cyc and pcDNA3luciferase (sample NIK+cgc), 1 μ g pcS3MTAlyNIK and pcDNA3luciferase (sample AlyNIK) and pcS3MTAlyNIK, pcDNA3cyc and pcDNA3luciferase (sample AlyNIK+cgc). pcS3MT AlyNIKNIK, pcS3MTNIK and pcDNA3cyc were used at a concentration of 1, 1, and 0.1 μ g/well respectively.

Please replace the paragraph beginning on page 18 at line 1 with the following amended paragraph:

Figure 11 shows the amino acid sequence of the intracellular domain of *cyc* (SEQ ID NO:1).

Please replace the paragraph beginning on page 18 at line 3 with the following amended paragraph:

Figure 12 shows the amino acid sequence of the 41 amino acid polypeptide from the membrane distal domain of *cyc* (41MDD) (SEQ ID NO:2).

Please replace the paragraph beginning on page 18 at line 6 with the following amended paragraph:

Figure 13 shows the nucleotide sequence of the intracellular domain of *cyc* (*cyc*ICD) (SEQ ID NO:5).

Please replace the paragraph beginning on page 18 at line 8 with the following amended paragraph:

Figure 14 shows the nucleotide sequence of the 41 polypeptide from the membrane distal domain of *cyc* (41MDD) (SEQ ID NO:6).

Please replace the paragraph beginning on page 18 at line 11 with the following amended paragraph:

Figure 15 shows the sequence of 12 aminoacids at the C-terminus of *cyc* involved in binding NIK (SEQ ID NO:3).

Please replace the paragraph beginning on page 19 at line 4 with the following amended paragraph:

The invention relates to the modulation of ~~IL-2~~ common gamma chain (*cyc*) and NIK interaction in pathologies involving said interaction.

Please replace the paragraph beginning on page 19 at line 17 with the following amended paragraph:

Multiple deletion mutants of both *cyc* and NIK were generated to define the binding domains in both proteins. The interactions were tested by yeast 2 hybrid tests and/or by immunoprecipitation studies (see examples below). Domains of *cyc* responsible for binding NIK were found in the membrane proximal domain (MPD) of *cyc* comprising 44 amino acid residues (from residue 282 to 325), named 44MPD (see SEQ ID NO: 17) and, ~~a~~ in a membrane distal domain (MDD) comprising 41 amino acid (from residues 329 to 369), named 41MDD (see SEQ ID NO: 2 and Figure 12). When 12 amino acids at the end of *cyc* (*cyc* residues 358-369, Fig 15 SEQ ID: NO 3 nucleotide sequence in SEQ ID NO: 4) were deleted from the intracellular domain of

cyc (cycICD), the binding to NIK decreases by 50% indicating that these residues play a major role in binding.

Please replace the paragraph beginning on page 20 at line 11 with the following amended paragraph:

cyc and NIK interaction was shown to be functionally significant. Reporter gene assays showed that cyc modulates NIK-induced NF- κ B activation. It is possible, under experimental conditions, to induce NF- κ B activation by overexpressing NIK. Activation of NF- κ B can be monitored in cells transfected with a construct encoding ~~luciferase~~ luciferase under the control of an NF- κ B inducible promoter. Using this luciferase system, NF- κ B activation was monitored in cells overexpressing NIK alone or together with different concentration of cyc (for details see examples below). It was found that modulation of NF- κ B depends on the concentration of NIK vis a vis the concentration of cyc within the cells (NIK/cyc). For example, enhancement of NIK mediated NF- κ B activation was observed when NIK/cyc was above 1 while inhibition of NIK mediated NF- κ B activation was observed when NIK/cyc was about equal or below 1.

Please replace the paragraph beginning on page 21 at line 12 with the following amended paragraph:

Progressively C-terminus deleted ~~cyc-at the distal membrane domain~~, 1-357, 1-341, 1-325, 1-303, were tested for their ability to modulate NF- κ B mediated by NIK in the luciferase system. For this purpose luciferase expression and activation of NF- κ B was measured in transfected cells overexpressing NIK and cyc or cyc deleted mutants at a ratio of about 1. Under these conditions cyc inhibits NF- κ B activation induced by NIK. It was found that full length cyc and fragments 1-357, and 1-341 were able to inhibit NIK mediated NF- κ B activation while mutants lacking the NIK binding domain such as fragments 1-325 and 1-303 did not have any effect on the activity of NIK mediated NF- κ B activation. The lack of effect of 1-325 and 1-303 confirms the involvement of the membrane distal domain of cyc-NIK interaction and the role of this interaction in NF- κ B modulation.

Please replace the paragraph beginning on page 22 at line 17 with the following amended paragraph:

The results obtained revealed that signalling ~~through~~ through cyc involves NIK and recruitment of signalosome proteins and consequently modulation of NF- κ B. Therefore

fragments of NIK, for example those comprising the C-terminus (from residue 624 to 947) and NIK640-720 the cyc-binding domain could be used to modulate signalling ~~through~~ through cyc.

Please replace the paragraph beginning on page 22 at line 21 with the following amended paragraph:

As mentioned above, interaction of endogenous NIK and cyc was demonstrated in peripheral mononuclear blood cells. It was found that in mononuclear cells NIK is constitutively associated with cyc, and upon IL-2 induction the signalosome components IKK-1, IKK-2, and IKK-3 are recruited to the IL-2 receptor ~~through~~ through cyc. The IL-2 receptor common γ chain was found to bind to NIK at a different location, other than IKK-1 binding region. Similar results were obtained upon stimulation of the cells with IL-15.

Please replace the paragraph beginning on page 22 at line 17 with the following amended paragraph:

The signalosome components co-immunoprecipitated with cyc upon IL-2 stimulation was shown to be active in a kinase assay. Thus these results demonstrate that under physiological conditions, binding of endogenous cyc to NIK

occurs, and that this interaction is involved in NIK activity and in NIK dependent NF- κ B activation. Therefore inhibiting the interaction of cyc and NIK may bring ~~to~~about inhibition of NF- κ B activation.

Please replace the paragraph beginning on page 24 at line 6 with the following amended paragraph:

The definition "functional derivatives" as herein used refers to derivatives which can be prepared from the functional groups present on the lateral chains of the amino acid moieties or on the terminal N- or C- groups according to known methods and are comprised in the invention when they are pharmaceutically acceptable i.e. when they do not destroy the protein activity or do not impart toxicity to the pharmaceutical compositions containing them. Such derivatives include for example esters or aliphatic amides of the carboxyl-groups and N-acyl derivatives of free amino groups or O-acyl derivatives of free hydroxyl-groups and are formed with acyl-groups as for example ~~alcanoyl~~alkanoyl- or aroyl-groups.

Please replace the paragraph beginning on page 24 at line 20 with the following amended paragraph:

The term "circularly ~~permuted~~permutated" as used herein refers to a linear molecule in which the termini have

been joined together, either directly or through a linker, to produce a circular molecule, and then the circular molecule is opened at another location to produce a new linear molecule with termini different from the termini in the original molecule. Circular permutations include those molecules whose structure is equivalent to a molecule that has been circularized and then opened. Thus, a circularly ~~permuted~~ permutated molecule may be synthesized de novo as a linear molecule and never go through a circularization and opening step. The particular circular permutation of a molecule is designated by brackets containing the amino acid residues between which the peptide bond is eliminated. Circularly ~~permuted~~ permutated molecules, which may include DNA, RNA and protein, are single-chain molecules, which have their normal termini fused, often with a linker, and contain new termini at another position. See Goldenberg, et al. J. Mol. Biol., 165: 407-413 (1983) and Pan et al. Gene 125: 111-114 (1993), both incorporated by reference herein. Circular permutation is functionally equivalent to taking a straight-chain molecule, fusing the ends to form a circular molecule, and then cutting the circular molecule at a different location to form a new straight chain molecule with different termini. Circular permutation thus has the effect of essentially preserving the

sequence and identity of the amino acids of a protein while generating new termini at different locations.

Please replace TABLE A on page 27 with the following amended TABLE A:

TABLE A Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser , <u>Ser</u> , Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser , <u>Ser</u> , Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

Please replace TABLE B on page 28 with the following amended TABLE B:

TABLE B More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser <u>Ser</u>	Ser <u>Ser</u>
Arg <u>Arg</u>	His, Lys, Arg
Leu	Ile, Phe, Met, Leu
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Met, Ile, Val
Gly	Gly
Ile <u>Ile</u>	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr <u>Tyr</u>	Phe <u>Phe</u> , Tyr
Cys	Ser, Cys
His	Arg, Gln, His
Gln	Glu, His, Gln
Asn	Asp, Asn
Lys	Arg, Lys
Asp	Asn, Asp
Glu	FLN <u>Gln</u> , Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

Please replace TABLE C on page 29 with the following
amended TABLE C:

TABLE C Most Preferred Groups of Synonymous Amino Acids

Appln. No. 10/511,314
 Amdt. dated October 11, 2007
 Reply to Office action of April 11, 2007

Amino Acid	Synonymous Group
Ser <u>Ser</u>	Ser <u>Ser</u>
Arg <u>Arg</u>	Arg <u>Arg</u>
Leu	Ile, Met, Leu
Pro	Pro
Thr <u>Thr</u>	Thr <u>Thr</u>
Ala <u>Ala</u>	Ala <u>Ala</u>
Val	Val
Gly	Gly
Ile <u>Ile</u>	Ile, Met, Leu
Phe <u>Phe</u>	Phe <u>Phe</u>
Tyr <u>Tyr</u>	Tyr
Cys	Ser, Cys
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Ile, Leu, Met
Trp	Trp

Please replace the paragraph beginning on page 31 at line 11 with the following amended paragraph:

The term "hybridization" as used herein shall include any process by which a strand of nucleic acid joins with complementary strand through a base pairing (Coombs J, 1994, Dictionary of Biotechnology, ~~stockton~~Stockton Press, New York NY). "Amplification" is defined as the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction technologies well known in the art (Dieffenbach and Dveksler, 1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY).

Please replace the paragraph beginning on page 32 at line 2 with the following amended paragraph:

where M is the molarity of monovalent cations, %GC is the percentage of G and C nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. For each ~~1-1~~1°C that the T_m is reduced from that calculated for a 100% identity hybrid, the amount of mismatch permitted is increased by about 1%. Thus, if the T_m used for any given hybridization experiment at the specified salt and formamide concentrations is ~~10-10~~10°C below the T_m calculated for a 100% hybrid according to the equation of Meinkoth, hybridization will occur even if there is up to about 10% mismatch.

Please replace the paragraph beginning on page 32 at line 10 with the following amended paragraph:

As used herein, "highly stringent conditions" are those which provide a T_m which is not more than ~~10-10~~10°C below the T_m that would exist for a perfect duplex with the target sequence, either as calculated by the above formula or as actually measured. "Moderately stringent conditions" are those, ~~which~~ that provide a T_m, which is not more than ~~20-20~~20°C below the T_m that would exist for a perfect duplex with the target sequence, either as calculated by the above formula

or as actually measured. Without limitation, examples of highly stringent (~~5-10°C~~ 10°C below the calculated or measured T_m of the hybrid) and moderately stringent (~~15-20°C~~ 20°C below the calculated or measured T_m of the hybrid) conditions use a wash solution of 2 X SSC (standard saline citrate) and 0.5% SDS (sodium dodecyl sulfate) at the appropriate temperature below the calculated T_m of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those ~~which~~ that allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 X SSC (or 6 X SSPE (standard saline-phosphate-EDTA), 5 X Denhardt's reagent, 0.5% SDS, 100 ~~micro~~ µg/ml denatured, fragmented salmon sperm DNA at a temperature approximately 20 to ~~25°C~~ 25°C below the T_m. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC (Ausubel, 1987, 1999).

Please replace the paragraph beginning on page 35 at line 8 with the following amended paragraph:

Thus, conjugating peptides, proteins or oligonucleotides to molecules that are known to bind to cell surface receptors will enhance membrane permeability of said peptides, proteins or oligonucleotides. Examples ~~for~~ of suitable groups for forming conjugates are sugars, vitamins, hormones, cytokines, transferrin, asialoglycoprotein, and the like molecules. Low et al., USP 5,108,921, describes the use of these molecules for the purpose of enhancing membrane permeability of peptides, proteins and oligonucleotides, and the preparation of said conjugates.

Please replace the paragraph beginning on page 40 at line 5 with the following amended paragraph:

The anti-Id Mabs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated.

_____The term "monoclonal antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and ~~F-(ab')₂~~, F(ab')₂, which are capable of binding antigen. Fab and ~~F-(ab')₂~~ F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

Please replace the paragraph beginning on page 40 at line 25 with the following amended paragraph:

The definition of "pharmaceutically acceptable" is meant to encompass any carrier, ~~which~~ that does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, the active protein(s) may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

Please replace the paragraph beginning on page 41 at line 11 with the following amended paragraph:

The invention relates to a method for the treatment of a disease involving signalling of a cytokine ~~through~~ through ~~IL-2~~ cyc in the pathogenesis of said disease comprising administration of a therapeutically effective amount of specific antibodies able to recognise and bind cyc protein and/or to fragments of cyc comprising regions responsible for binding NIK such as 41MDD and 44MPD, 1-357 and 1-341, to a subject in need.

Please replace the paragraph beginning on page 42 at line 25 with the following amended paragraph:

In addition, screening for molecules generated by combinatorial chemistry, ~~which~~ that inhibit NIK and IL-2 receptor ~~γ~~ chain ~~receptor~~-interaction comprising a polypeptide comprising the intracellular domain of the cγc or a mutein, fusion protein, functional derivative, active fraction, circularly permutated derivative or fragment thereof, comprising: coating or capturing (by a specific antibody bound to the plate) one of the proteins (e.g. NIK or NIK640-720) in a plate and detecting the binding of the other protein (e.g. cγc, ICDcγc or fragments thereof) bound to the plate with specific antibody in the presence or absence of organic compounds.

Please replace the paragraph beginning on page 44 at line 5 with the following amended paragraph:

More than 5000 clones appeared on the selection plates. About half of the resistant clones were analyzed by α-gal assay and approximately 60% of them turned out positive with varying intensity of blue colour. Plasmids were isolated and purified from 800 colonies. The DNA inserts of 400 plasmids out of the 800 (chosen according to colour intensity which is indicative of affinity of binding) were amplified, by

polymerase chain reaction (PCR) using primers corresponding to the flanking sequences of the inserts in the cDNA library, and sequenced. Most of the preys detected~~7~~ turned out to be non-specific, e.g.: 80% of the DNA inserts corresponded to 3' and 5' untranslated regions of various genes and 10% to DNA inserts encoding immunoglobulins. The remaining 10% corresponded to segments encoding regions of proteins. Some of the positive colonies turned blue 4-8 days after ~~seeded~~ seeding, some after about 8-12 days, and others became coloured late, up to 12-16 days after seeding. The speed of the colour development in positive colonies is indicative of the strength of protein-protein interaction, i.e. the faster the colour appears, the stronger the interaction.

Please replace the paragraph beginning on page 44 at line 18 with the following amended paragraph:

One of the binding proteins found, the IL-2 receptor gamma chain~~-receptor~~, was chosen for further analysis. The IL-2 receptor gamma chain ~~receptor~~ is a subunit of the IL-2, IL-4, IL-7, IL-9, IL-13, IL-15 and IL-21 receptor complexes~~7;~~ therefore, it is commonly dubbed as the 'common γ chain' (γ c).

Please replace the paragraph beginning on page 44 at line 24 with the following amended paragraph:

The detection of a specific interaction between two different mammalian proteins in a two-hybrid system in yeast does not necessarily imply that there exists a corresponding interaction between the proteins in a native mammalian environment. Therefore, in order to verify NIK and *cyc* interaction in a mammalian environment, co-immunoprecipitation studies of NIK and *cyc* were carried out in lysates of 293-T cells ~~overexpressing~~ overexpressing these proteins (for details see Example 9).

Please replace the paragraph beginning on page 46 at line 22 with the following amended paragraph:

The deletion mutants were created by sequentially ~~introduction~~ introducing ~~of~~ stop codons in the cytoplasmic domain of *cyc*, in gaps of 10-20 amino acids. The DNA encoding the full-length *cyc* or its deletion mutants were introduced into the pGADT7 prey vector (Clontech) for testing their binding to NIK in the SFY526 heterologous yeast strain by the two hybrid assay. The SFY526 yeast strain is prototrophic for TRP and Leu. pGBKT plasmids (bait vector) have the Trp1 wild type gene and pGAD has the wild type Leu2 gene. Thus, only doubly transfected yeast will grow on selective Leu Trp media. Functional GAL4 will be restored in doubly transfected yeast when the chimeric proteins fused to GAL4 domains interact,

bringing the activation domain and DNA binding domain of GAL4 to close proximity. The level of LAC-Z expression is indicative of the strength of the protein-protein interaction. Lac-Z activity was assessed by the standard beta-gal/colony lift filter assay (Clontech, Yeast Protocol Handbook, Chapter VI).

Please replace the paragraph beginning on page 47 at line 7 with the following amended paragraph:

Since introduction of *cyc* and mutants into the pGADT7 prey vector for assessing their binding to NIK as bait manifested high non-specificity, the interactions were tested in the reverse orientation: i.e. deletion mutants were cloned into the bait vector and NIK or C-terminus of NIK (residues 624-947) in the ~~prey-prey~~ vector. The results summarized in Table 1 show that none of the deletions, ~~but~~ except the cytoplasmic domain of *cyc* (ICD) alone, showed strong binding, to both NIK and NIK C-terminus. The binding of most of the ICD (lacking 5 amino acid from its proximal membrane domain) to both NIK and C-terminus NIK was stronger than that of the full-length *cyc* molecule. A 50% reduction in affinity to NIK was observed by deleting 12 amino acids or 44 amino acids at the membrane distal end of *cyc*ICD.

Please replace Table 1 beginning on page 47 at line 16 with the following combined Table 1:

Table 1.

cyc amino acid residues	NIK624-947 (C-terminal domain)	NIK	Lamin
Full length (1-369)	+/-	-	-
1-357	-	-	*
1-325	-	-	*
1-303	-	-	*
1-282	-	-	*
289-369 (most of ICD)	++++	+++	-
289-357 (12 aa deleted from the membrane distal domain)	++	*	*
289-325 (44 aa deleted from the ICD)	++	*	*

* Not tested

Please replace the paragraph beginning on page 49 at line 1 with the following amended paragraph:

The binding of 41 MDD polypeptide to full length NIK or C-terminus NIK was tested in both orientations (i.e. 41 MDD

as the ~~prey~~prey and NIK as the bait and vice versa). The results obtained are shown in Table 2. The interaction is relatively weak when NIK serves as the prey partner, but strong when NIK serves as the bait. The interaction of the 41 MDD is stronger with the C-terminus of NIK than with the full length NIK. These results confirmed that the 41 MDD polypeptide is involved in binding to NIK.

Please replace the heading prior to the paragraph beginning on page 56 at line 6 with the following amended heading:

**Effect of *cyc* in modulating signal transduced ~~through~~
through the LT β receptor:**

Please replace the paragraph beginning on page 56 at line 6 with the following amended paragraph:

Induction of the LT β receptor by its ligand, results in NF- κ B activation. It is suggested in the literature that NIK participates in signaling ~~through~~through the LT β receptor. Thus, the effect of overexpressing the whole cytoplasmic *cyc* polypeptide or the 41 distal domain (329-369) on NF- κ B activation mediated by the LT β receptor was tested. Activation of NF- κ B was monitored by the luciferase reporter assay (for details see Example 10).

Please replace the paragraph beginning on page 56 at line 12 with the following amended paragraph:

A cell line was prepared from mouse embryonic fibroblast cells, which are generally known to express the LT β receptor. 10⁵ cells of the above line were seeded per well in 6 well plates. 24 hours later transfection was performed (with Gene porter transfection reagent, Gene therapy systems) with the plasmid pcGST ~~ICe β~~ -ICDcyc expressing the intracellular domain of cyc (cyc ~~ICD~~ICD) fused to GST or with pcGST41MDD expressing the 41 distal domain of cyc fused to GST and the expression plasmid encoding luciferase reporter protein under the control of an NF- κ B inducible promoter (pcDNA3 luciferase). NF- κ B activation was measured indirectly by measuring the luciferase activity present in the cells.

Please replace the paragraph beginning on page 56 at line 20 with the following amended paragraph:

Total DNA concentration was normalized to 2 μ g/well with empty vector (pcDNA3). pcGST ~~ICe β~~ -ICDcyc and pcGST41MDD were used at a concentration of about 1 μ g/well. 24 hours after the transfection, cells were stimulated with 50ng/ml recombinant LT β (cat# L-5162, Sigma) for 1 hour.

Please replace the paragraph beginning on page 56 at line 27 with the following amended paragraph:

The above results suggest that *cyc* may be involved in signaling ~~through~~ through the LT β receptor. The *cyc* 41 distal domain inhibits signaling ~~through~~ through LT β receptor, indicating that this polypeptide or fragments thereof may serve as candidates for peptide based drug designing. Such drugs may modulate NIK action and therefore are valuable in preventing or alleviating inflammatory responses or in modulatory immunoregulatory processes.

Please replace the paragraph beginning on page 57 at line 6 with the following amended paragraph:

The binding region in NIK was determined by testing the interaction of a series of NIK deletion mutants with *cyc* employing the yeast two-hybrid system. The truncated mutants of NIK were cloned into the pGBT9 two-hybrid bait vector and *cyc* was cloned into the pGADT7 prey vector. The binding was tested in the SFY526 heterologous yeast strain, by beta-gal assay. The results are summarized in Table 4.

Please insert the heading prior to the table beginning on page 57 at line 12:

Table 4.

Please replace the paragraph beginning on page 57 at line 15 with the following amended paragraph:

To define more precisely the domain of NIK responsible for binding *cyc*, more deletion mutants of NIK were created and their binding to *cyc* was analysed by co-immunoprecipitation. 293T cells were transfected with vector encoding *cyc* and His tagged NIK deletion mutants and the binding of the different deletion mutants to *cyc* was tested by coimmunoprecipitation (see details in Example 9). Antibody against the *cyc* was used for immunoprecipitation and anti His antibodies were used to detect His-NIK deletion mutants of the immunoprecipitated material on Western blots. The results are summarized in ~~Table 4~~Table 5.

Please replace the Table name beginning on page 58 at line 7 with the following amended Table name:

~~Table 4~~**Table 5.**

Please replace the paragraph beginning on page 58 at line 16 with the following amended paragraph:

The two-Hybrid system used for screening was the ~~Matchmaker~~MATCHMAKER version III (Clontech). In this system the bait gene (NIK gene) is expressed as a fusion to the GAL4 DNA binding domain (DNA-BD), while the ~~pray~~prey genes or cDNA

library is expressed as a fusion to the GAL4 activation domain (AD). When the DNA-BD and AD are brought into proximity, transcription of four reporter genes is activated (encoding HIS, ADE, lacZ and α -gal).

Please replace the paragraph beginning on page 59 at line 6 with the following amended paragraph:

Clones ~~growing~~ are grown on plates under high stringency conditions, i.e. in plates without LEU (selection marker for the bait encoding plasmid), TRP (selection marker for the ~~pray~~ prey encoding plasmid), HIS and ADE and impregnated with substrates for detection of α -gal expression. Plasmids were purified from positive clones by lysis of the yeast cells (with detergent and mechanical stress) followed by phenol extraction and ethanol precipitation of the DNA. cDNA inserts in the plasmids were amplified by PCR with flanking primers specific for the library vector pACT2. Individual amplified cDNAs were directly cloned into a mammalian expression vector for further biochemical analysis.

Please replace the paragraph beginning on page 62 at line 6 with the following amended paragraph:

NIK and cyc interaction was demonstrated in a mammalian cell environment, in lysates of 293-T cells

~~overexpressing~~ overexpressing these proteins (see Example 4).

The following experiment was carried with endogenous proteins, in cells naturally expressing these proteins. Thus, peripheral blood mononuclear cells (PMBC) (500×10^6 cells) were incubated with IL-2, lysed and immunoprecipitated with anti cyc antibodies (for immunoprecipitation see Example 9). Co-immunoprecipitated proteins bound to cyc were detected in Western blots using relevant antibodies. The candidate proteins tested for co-immunoprecipitation with cyc were those proteins normally present in the signalosome, such as NIK, IKK α (IKK-1), IKK β (IKK2), IKK γ (NEMO). The co-immunoprecipitated proteins were tested in lysates of cells tested at time-0 and after four-hour incubation with IL-2. The results summarized in Figure 16 A show that NIK is coprecipitated with cyc before and after stimulation with IL-2. Therefore NIK was found constitutively associated with cyc. Traces of IKK-1 was found in the basal level and upon 4 hours incubation with IL-2, other signalosome components, i.e. IKK-2 and NEMO, were recruited to the IL-2 receptor ~~through~~ through the cyc. The results indicate that the IL-2 receptor ~~common~~ gamma chain is bound to NIK at a different location than the IKK-1 binding region. Similar results were obtained upon stimulation of the cells with IL-15 (figure 16A right panel).